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Research Article



Effects of Dietary Supplementation of Date Palm (*Phoenix dactylifera*) on the Growth Performance, Carcass Characteristics, and Immune Response of Broiler Chicken

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ABSTRACT

Introduction: Phytogenic substances encompass materials sourced from plants, including spices, herbs, essential oils, and various plant extracts. These materials have been employed for millennia as natural remedies, owing to their distinctive properties. The present study aimed to evaluate the effects of date palm (Phoenix dactylifera) seed powder supplementation on growth performance, carcass traits, and immune response of broiler chickens.

Materials and methods: A total of 250 one-day-old broiler chicks of mixed sex (Ross 307) were randomly distributed into five treatments with five replicates. The basal diet was formulated to meet broiler chickens' nutrient requirements. Chickens in treatment 1 (T1) received a basal diet, while T2, T3, T4, and T5 were fed with the same diet of date palm seed powder at 100 g, 200 g, 300 g, and 400 g per kg, respectively. Chickens had unlimited access to feed and clean water throughout the 42 days of the experimental period. Parameters, including growth performance, carcass characteristics, and immune response of broiler chickens were examined during the study. Phytochemical examination indicated that date palm seed powder contained phenol at 340.92 mg/g followed by flavonoids (205.67 mg/g), terpenoids (112.81 mg/g), tannins (96.73 mg/g), saponins (41.64 mg/g), alkaloids (30.57 mg/g), and steroids (21.68 mg/g).

Results: The treatment significantly influenced average daily weight gain, average daily feed intake, and feed conversion ratio. Chickens fed with date palm seed powder had higher average body weight, feed conversion ratio, and feed intake than the control group. Dressing percentage, slaughtered weight, and edible organ weights were higher in T5 than in other groups.

Conclusion: Date seed powder can be incorporated into broiler chickens' diets up to 400g/kg without negative impacts on the chickens' performance.

1. Introduction

Phytogenic includes materials derived from plants, such as spices, herbs, essential oils, and other plant extracts. They have been daily utilized for thousands of years and as natural treatments due to certain qualities1. Due to their wide variety of efficacies and the impacts on sustainability and safety, phytogenic demonstrated a wider range of activities in animal nutrition compared with synthetic chemicals^{2,3}. Phytoconstituents, including phenols, tannins, terpenoids, saponins, alkaloids, and flavonoids that are obtained from medicinal plants are safe, efficacious,

environmentally benign, and capable of halting the spread of antibiotic resistance^{4,5}. Plant roots, seeds, stem bark, flowers, and leaves contain various quantities of phytoconstituents or bioactives⁶. The composition of herbs reveals differences influenced by their growth stage, geographical location, climate, and storage conditions, thereby elucidating their effectiveness⁶. Numerous pharmacological and therapeutic actions, including antiinflammatory, antioxidant, antifungal, anti-helminthic, and immune-stimulatory, can be carried out by bioactive

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substances4.

Powdered date palm (Phoenix dactylifera) seed is one of the prospective phytogenic that is essential for animal nutrition⁷. The Pheonix dactylifera is monocotyledonous, drought-resistant plant that is in the Aracaceae family⁷. Numerous species of the date tree can be found widely throughout the Canary Islands, Northern Africa, Arabia, and southwest Asia, all the way to Pakistan and India8. Due to the high content of macro and micronutrients, such as vitamins and amino acids, date fruits became popular as a major food item worldwide8. There are the following nutritional values in date seeds, including 71.9-73.4 % carbohydrates, 5.0-6.3% protein, 9.9-13.5 % fats, 6.4-11.5 % fiber, and 1.0-1.8 ash. The seeds are rich in potassium, phosphorus, calcium, magnesium, manganese, zinc, copper, nickel, cobalt, sodium, and chromium7. They are also odorless and taste somewhat bitter when combined9. Numerous phytochemicals, including phenolic compounds, flavonoids, terpenoids, alkaloids, and saponins, are abundant in date palm seeds and have a variety of pharmacological effects, such as antiinflammatory, antimicrobial, antioxidant, hepatoprotective, antidiabetic, and antiviral effects^{9,10}. The seeds have the potential to be highly effective nutritional therapeutic agents for treating a variety of chronic illnesses. They can also inhibit the growth of certain highly pathogenic bacteria, including Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, and Enterococcus faecalis^{11,12}.

Previous studies demonstrated that adding phytogenic to the diet of broilers can affect endogenous enzyme activity and nutrient absorption¹³, inhibit the growth of certain virulent diseases, and have a limited impact on beneficial bacteria¹⁴, enhance blood parameters, and scavenge free radical activity¹⁵. There is limited information regarding the potential benefits of date palm seed powder on broiler chicken performance.

The current study assessed the impact of date palm seed powder on the immune system, carcass features, and growth performance of broiler chickens. The present study can address the growing number of antibiotic-resistance instances also enhancing broiler performance, profit margin, and quality feed and animal-derived products.

2. Materials and Methods

2.1. Ethical approval

The present study have been done in the poultry section of Sumitra Research Institute, Gujarat, located between 28° 20' N and 75° 30' East India. All experimental guidelines and procedures were approved by the ethics committee of the Animal Nutrition and Biochemistry Department of the Institute (AF/2022T/008). The trial was carried out between January to March 2022.

2.2. Collection and processing of date palm seed powder

Fresh dates were harvested within Sumitra Research

Institute's environment and sent to the crop protection department for proper authentication by a certified taxonomist before it was assigned an identification number (DF/2022A/DSP). Thereafter, seeds were manually removed, washed with running tap water, and sundried for 10 days until seeds turned light brown and a constant weight was achieved. Dried date palm seeds were blended into powder with an electric blender and stored in an airtight labeled container for further examination in the laboratory immediately.

2.3. Animal management, experimental treatments, and design

A total of 250 one-day-old mixed-sex broiler chicks (Ross 307) were purchased from a reputable breeding farm in Gujarat, India. Chicks with an average initial body weight of 50.88 ± 0.38 g were reared in a galvanized battery cage kept in semi-closed pens (150 cm \times 100 cm \times 85 cm). Before the commencement of the actual experiment, the pens $(4m \times 2.5 \text{ m})$, watering, and feeding troughs were thoroughly washed and sprayed, disinfected with Morigad Plus® (Netherlands), and the room was fumigated using mixing potassium permanganate powder with formalin 10 % solution. A foot bath was also provided at the entrance of the pen to ensure proper biosecurity. Chickens were randomly divided into five dietary treatments and five replications per treatment having 10 chicks per replicate. The experimental design adopted a fully randomized approach. The chicks were brooded using 200-watt bulbs as sources of heat and light for each pen to ensure adequate and uniform distribution of heat and light. The lightning program was scheduled for 24-hour constant light with an initial temperature of 36°C which was reduced weekly to 2°C until it attained 27°C. Chicks were fed on a basal diet (Corn-soya based) formulated according to the recommendation of the NRC16 (Table 1). Chickens in treatment 1 (T1) were fed on a basal diet with 0.30 g neomycin (according to the manufacturer's recommendation) per kilogram of feed while those in treatments 2, 3, 4, and 5 (T2,3,4, and 5) were fed with the same diet with date palm seed powder at 100 g, 200 g, 300 g, and 400 g per kilogram diet. Feeding and watering troughs were cleaned daily and chicks were vaccinated according to the schedule designed for broilers at Sumitra Research Institute, Gujarat, India (Table 2). The experiment lasted for 42 days and all other management practices were strictly observed throughout the experiment.

2.4. Experimental measurements 2.4.1. Growth performance parameters

Feed consumption or intake in grams was determined at the end of the experiment (day 42) as the difference between the feed offered and refused. Average weight gain was determined by subtracting the average initial weight of chickens from their final body weight. Average daily weight gain was calculated as average weight gain to the number

of experimental days. Average daily feed intake was estimated by dividing the total feed intake by the number of experimental days. The feed conversion ratio (feed consumed to produce a unit of gain) was computed as the ratio of average feed consumption to average body weight gain⁴. The weight of the chickens was determined with a sensitive scale (Model - DFS-A -6111Y, China).

2.4.2. Phyto-constituents in date palm seed powder

Quantification of phyto-constituents in date palm seed powder was carried out according to the recently published method outlined^{17,18}. Each phytochemical component or phyto-constituents was determined using thermo-specific TSQ 9000 (Netherlands) triple quadrupole gas chromatography-mass spectrometry (GC-MS) system at different optical densities (alkaloids, 500 nm), flavonoids (460 nm), terpenoids (370 nm), alkaloids (550 nm), tannins (480 nm), steroids (250 nm), saponins (360 nm) and phenols (670 nm). The machine has the following technical specifications; inlet temperature of 450°C, column temperature (4-450°C, pressure range (0-100 psi ± 0.002 psi) and heating rate up to 1201/min while the mass spectrometer unit is adjusted at an ion source temperature (100-350°C), stability (± 0.10 amu/48 hours), mass range (1.5-1000 amu), scan rate (up to 10000 amu/sec) and ionization energy (5 eV-250 eV).

2.4.3. Carcass evaluation

At the end of the experiment, four chickens from each replicate were randomly selected for carcass evaluation. The selected chickens were starved for twelve hours and weighed before slaughtering mechanically with a sharp knife according to the method outlined by Shung et al.¹³. The chickens were humanly slaughtered by severing the jugular vein, followed by dipping into hot water and defeathering mechanically. Carcasses were manually eviscerated suspended from the evisceration line and allowed to drain for 15 minutes before weighing. The dressing percentage was calculated as the proportion of live weight to slaughter weight multiplied by 100.

2.4.4. Proximate composition of date palm seed powder

Analysis of feed was done using a commercial kit, NIRSTM DA1650 (Netherlands) feed analyzer with the following technical specifications, wavelength range (1100 to 1650 nm), ambient temperature (5-40°C), ambient humidity (less than 93% RH), and results was generated at an analysis time of less than 1 minutes.

2.5. Immune response examination on Newcastle disease

At the end of day 42, blood samples were collected from four randomly selected chickens per replicate for an immunity test against Newcastle disease. Blood was collected via the wing vein into a sterile, labeled sample bottle. After collection, it was kept in an ice pack to be sent to the laboratory for further examination. Analysis was carried out using CypherOne hemagglutination analyzer (USA). Collected blood samples were placed in a prepared hemagglutination plate, CypherOne hemagglutination analyzer captures an image of the plate and uses an automated algorithm to determine the best endpoint titer value of each dilution series. Results are automatically displayed with the high-resolution image and saved in a database which may be reviewed and later recorded⁶.

2.6. Analysis of data

All collected data were subjected to one-way ANOVA using Statistical Package for Social Sciences, version 21. The differences among the treatment means were determined (p < 0.05) by Duncan's multiple-range test of the same statistical package.

3. Results

The composition and nutrient levels of the basal diet are presented in Table 1. Broiler's starter mash contained crude protein of 23.09%, crude fiber (3.47%), ether extract (3.08%), calcium (1.42%), phosphorus (0.51%), and metabolizable energy of 2927.6 kcal/kg. Finisher's mash contained metabolizable energy (3190.6 kcal/kg), crude protein (21.11 %), crude fiber (4.39%), ether extract (3.46%), calcium (1.63%), and phosphorus (0.72%). As Figure 1 presents, Phyto-constituents of date palm seed powder, revealed that phenol (340.92 mg/g) was one of the major secondary bioactive compounds followed by flavonoids (205.67 mg/g), terpenoids (112.81 mg/g), tannins (96.73 mg/g), saponins (41.64 mg/g), alkaloids

Table 1. Composition and nutrient levels of basal diet fed to Ross 307 broilers from day 0-42

	Ctt	Pinish
	Starter mash	Finisher mash
Items	(0-28 d)	(29 - 42 d)
	Content (%)	Content (%)
Corn	52.00	57.00
Soya bean meal	36.00	30.00
Fish meal	5.00	4.81
Calcium carbonate	2.00	2.50
Di-calcium phosphate	4.00	4.52
Lysine	0.20	0.25
DL-Methionine	0.25	0.25
1,2*Premix	0.25	0.25
Salt	0.30	0.32
Total	100.00	100.00
Determined nutrient levels (%)		
Crude protein	23.09	21.11
Crude fibre	3.47	4.39
Ether extract	3.08	3.46
Calcium	1.42	1.63
Phosphorus	0.51	0.72
Energy (metabolizable) (Kcal/kg)	2927.6	3190.6

Mineral-vitamin premix, each 2.5 kg sachets contains; Thiamine, 8000 mg, riboflavin, 12,000 mg, pyridoxine, 5000 mg, cyanocobalamine, 5000 mg, niacin, 20,000 mg, D-panthotenate, 10,000 mg, folic acid, 500 mg, biotin, 2000 mg, cholecalciferol, 3,000,000 iu., tocopherol acetate, 25,000 iu., ascorbic acid, 62,000 mg, manganese, 56mg, iron, 70,200 mg, 300 mg, iodine, 200 mg, selenium, 85 mg, choline chloride, 46,000 mg

Table 2. Vaccination schedule for Ross 307 broiler chicken between 0-42 days

Vaccination	Day(s) administered	Route of administration
Newcastle disease vaccine (Italy) 1st dose	7	Oral
Newcastle disease vaccine (Italy) booster dose	14	Oral
Infectious bursal disease vaccine (Netherlands, 1st dose)	18	Oral
Infectious bursal disease vaccine (Netherlands, 1st dose)	24	Oral

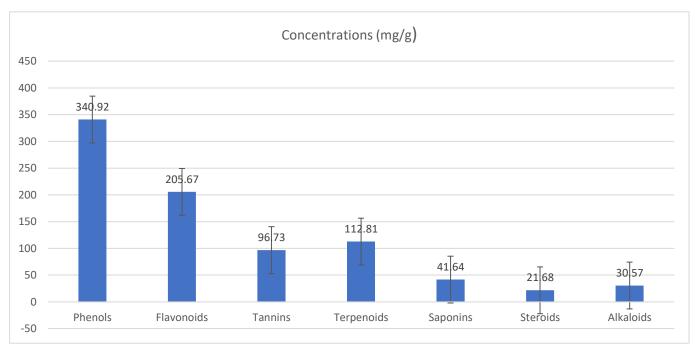


Figure 1. Phyto-constituents (mg/g) in date palm seed powder

(30.57 mg/g), and steroids (21.68 mg/g). As can be seen in Table 3 the effect of dietary supplementation of date palm seed powder on the growth performance of broiler chickens on day 42 is presented. Treatments had a significant impact on the average daily weight gain, average daily feed intake, feed conversion ratio, and mortality rate (p < 0.05). However, average daily weight gain was higher among chickens fed T5 (400 g date palm powder/kg diet), intermediate among chickens that received T2 (100 g date palm powder/kg diet), T4 (300 g date palm powder/kg diet, p > 0.05), and lowest in T1 (p < 0.05). The mortality rate was higher in T1 (2.41%) followed by T2 (1.52%, p < 0.05). None was recorded among chickens in T 3, 4, and 5 (p > 0.05). Carcass characteristics of broiler

chickens fed a diet supplemented with date palm seed powder on day 42 are presented in Table 4 revealing that slaughtered weight, dressing percentage, and percentage of cut parts from wings, breast, drumstick, thigh, gizzard, liver, heart, and proventriculus were influenced by the treatments (p < 0.05). Following a similar pattern, all parameters were measured, as values were higher in T4, intermediate among chickens that received T2, 3, and 4, and the lowest in T1. As can be seen in Table 5, antibody titer for Newcastle disease immune response (at days 21 and 42) of broilers fed the diet supplemented with date palm seed powder. Although the chickens fed with T2, 3, 4, and 5 had similar values, they were significantly higher than those in T1 (p > 0.05).

Table 3. Effects of date palm seed powder on the growth performance of broiler chickens at day 42

Variables	1	2	3	4	5	SD
Average initial weight (g)	51.26	50.88	51.1	51.02	51.11	0.2
Average final weight (g)	1880.3c	2320.6b	2339.7b	2400.5b	2617.5a	42.04
Average weight gain (g)	1829.04c	2269.7b	2288.6b	2349.5ь	2566.4a	38.02
Average daily body weight gain (g)	43.55c	54.04b	54.49b	55.94ь	61.10a	0.42
Average total feed intake (g)	4261.2b	4608.5a	4711.3a	4718.5a	4710.6^{a}	61.43
Average daily feed intake (g)	101.5b	109.7a	112.2a	112.3a	112.1a	0.81
Feed conversion ratio	2.32a	2.03b	2.05b	2.00^{b}	1.83c	0.16
Mortality (%)	2.41a	1.52 ^b	0	0	0	0.02

SD: standard deviation; 1: Basal diet; 2: Basal diet supplemented with 100 g date palm seed powder per kg; 3: Basal diet supplemented with 200 g date palm seed powder per kg; 4: Basal diet supplemented with 300 g date palm seed powder per kg; 5: Basal diet supplemented with 400 g date palm seed powder per kg; abc Means on the same row having different superscripts are significantly different (p < 0.05)

Table 4. Effects of palm seed powder on the carcass characteristics of broiler chicken on day 42

Variables	1	2	3	4	5	SD
Live weight (g)	1716.7c	2100.3ь	2100.5ь	2340.6ь	2517.9a	39.07
Slaughtered weight (g)	1196.7c	1550.3ь	1538.5b	1768 ^b	1937.8a	29.44
Dressing percentage (%)	69.70c	73.81 ^b	73.24 ^b	75.54 ^b	77.00a	0.15
Wing (%)	8.52c	10.45^{b}	10.52b	11.41 ^b	14.91a	0.04
Breast (%)	16.34c	21.56b	21.6b	23.71 ^b	28.65a	0.08
Drum stick (%)	4.33c	$6.07^{\rm b}$	6.11^{b}	7.21 ^b	9.05^{a}	0.02
Thigh (%)	7.47 ^c	$8.04^{\rm b}$	8.11 ^b	8.53b	10.42^{a}	0.02
Gizzard (%)	1.93c	2.04^{b}	2.14^{b}	2.65b	4.02a	0.01
Liver (%)	1.24c	1.62b	1.72b	1.77b	2.02a	0.01
Heart (%)	0.18^{c}	0.21^{b}	0.25^{b}	0.34^{b}	0.40^{a}	0.02
Proventriculus (%)	0.72c	0.91 ^b	0.98^{b}	1.01 ^b	1.34a	0.01

SD: standard deviation; 1: Basal diet; 2: Basal diet supplemented with 100 g date palm seed powder per kg; 3: Basal diet supplemented with 200 g date palm seed powder per kg; 4: Basal diet supplemented with 300 g date palm seed powder per kg; 5: basal diet supplemented with 400 g date palm seed powder per kg; abcMeans on the same row having different superscripts are significantly different (p < 0.05)

Table 5. Antibody titer for Newcastle disease immune response (at days 21 and 42) of broiler chickens fed diet supplemented with date palm seed powder

Newcastle disease antibody titer (Log 10)	1	2	3	4	5	SD
Day 21	4.92b	5.32a	5.56a	5.81a	5.92a	0.21
Day 42	5.01 ^b	6.07^{a}	6.18a	6.27a	6.61a	0.18

SD: standard deviation; 1: Basal diet; 2: Basal diet supplemented with 100 g date palm seed powder per kg; 3: Basal diet supplemented with 200 g date palm seed powder per kg; 4: Basal diet supplemented with 300 g date palm seed powder per kg; 5: Basal diet supplemented with 400 g date palm seed powder per kg; 3bMeans on the same row having different superscripts are significantly different (p < 0.05)

4. Discussion

According to Keppler et al. 19, medicinal plants contain therapeutic agents in varying concentrations in their leaves, stem barks, roots, flowers, and seeds. The therapeutic qualities or properties of these compounds are determined by their quantities. Phenolic compounds demonstrate antioxidant²⁰, anti-inflammatory²¹, cytotoxic, hypolipidimic, antibacterial, and mutagenic actions^{22.} Flavonoids have been claimed to exhibit anti-microbial, anti-inflammatory, immunestimulatory23, and hepato-protective effects²⁴. Terpenoids serve as an anti-diarrheal, anti-bacterial, and anti-inflammatory²⁵. Alkaloids demonstrate medicinal plants have analgesic and antimalarial properties²⁶. Saponins have antibacterial, anti-diarrhea, and gastro-protective qualities²⁷. Adewale et al.²⁸ reported that tannins have anti-inflammatory and anti-Additionally, qualities. microbial they antibacterial qualities to suppress the growth of certain microorganisms, including Salmonella, Escherichia coli, Staphylococcus species, Klebsia species, and Streptococcus species. Pharmaceutical companies can produce steroid hormones using steroids²⁹. They also immune-stimulatory and anti-inflammatory qualities²⁸. Animal performance and their products benefit from the synergistic combination of these bioactive substances²⁵. Moreover, it protects against the possible spread of antibiotic resistance to humans²⁷.

The current study has demonstrated that synthetic antibiotics, specifically neomycin, along with date palm seed powder, can influence the enzymatic activities within the gastrointestinal tract. It is worth to note that dietary supplementation of date palm seed powder at 400 g per kg diet (T5) can facilitate the activities of endogenous enzymes relative to the other treatment²⁸.

This results in efficient feed utilization and a reduction in the retention time of feed translating to better weight gain among chickens. Chickens in T1 (0.30 g neomycin per kg diet) had the lowest average daily weight gain compared to those that received T2 (100 g date palm seed powder per kg diet), T3 (200 g date palm seed powder per kg diet), and T4 (300 g date palm seed powder per kg diet). According to the obtained results, the presence of phyto-constituents in date palm seed powder (Table 3) in the diet creates a balanced gastrointestinal flora and suppresses the activities of pathogenic or harmful organisms. The observed average daily weight gain range in the present study (43.55 -55.94 g/bird) was in line with the result of Shittu et al.²⁹ who discovered that average daily weight gains of broiler chickens fed a diet supplemented with Sida acuta leaf extract varied from (41.88 - 57.00 g/b). The outcome was higher than 33.77 - 39.09 g/bird reported by Farhadi et al.30 when eucalyptus oil was fed to broilers at 0.5 mL per kg diet. The variation in results could be attributed to the difference in the inclusion levels as well as the nature of phyto-constituents in the test ingredient³¹. The average daily feed intake which varied from 101.5 - 112.1 g/b was higher (P < 0.05) in chickens that received date palm seed powder relative to T1. The result indicates that date palm seed powder has appetite-stimulating properties. The increased intake could be due to its flavor which in turn affects the feed conversion ratio of chickens. The obtained result was similar to the reports of Obadire³², who found out that the average daily feed intake of broiler chickens fed phytogenics ranged from 94.5-109.5 g/b. 2.41% and 1.52% mortality were recorded among chickens that received T1 and T2 respectively, while none was recorded in the other treatments. The results indicated the significant antibacterial and antimicrobial properties of date palm seed powder, which enables

them to curb the activities of pathogenic microorganisms in the gastrointestinal tract especially when supplemented in the diet of broiler chickens between 200-400 g/kg diet. The obtained results are in consonance with the reports of Alagbe et al.³³.

The increased supplementation of date palm seed powder especially at 400 g/kg diet led to a significant increase in dressing percentage relative to the other treatments (p < 0.05). The obtained results revealed that date palm seed powder contains some essential nutrients for the weight gain of chickens. The presence of phytoconstituents also promotes feed conversion efficiency in chickens³⁴. The dressing percentage observed in the current study which varied from 69.70-77.0 % was similar to reported values by Alagbe³⁵ when turmeric powder was supplemented in the diet of broiler chickens. Slaughtered weight which ranged from 1196.7-1937.8 g was lower in comparison with those reported by Alagbe et al.36 when an aqueous extract of Balanites aegyptiaca and Alchornea cordifolia stem bark mixture was fed to broiler chickens. The outcome of the study also revealed that the phyto-constituents in date palm seed powder are non-toxic and were within the permissible range for broiler chickens³².

Antibody titer against Newcastle disease on day 21 and 42 varied from 4.92 - 5.92 (Log10) and 5.01 - 6.61 (Log10) higher among chickens fed a diet supplemented with date palm seed powder and lower in T1. The result suggests that date palm seed powder possesses immunestimulatory properties due to the presence of secondary metabolites with pharmacological properties. These chemical compounds are capable of modulating cytokine production from macrophages or down/up-regulate the expression of the gene coding for cytokine production to keep inflammation under control or to support the development of acquired immunity³⁷⁻³⁹. The antibody titer range (4.92-5.92 [Log10]) on day 21 was in line with the result of a study by Nnadi et al.40, who discovered that antibody titer of chickens against Newcastle disease on day 21 fed diet supplemented with oregano essential oil. Similarly, Ratcliffe and Hartle⁴¹ reported an antibody titer that varied from [3.85-4.46 (Log10)] for the infectious bursal disease of broiler chickens fed a diet supplemented with phytogenic.

5. Conclusion

The date palm seed powder contains numerous phyto-constituents with medicinal value (antimicrobial, antifungal, hepato-protective, immune-stimulatory, antiviral, anti-inflammatory, antioxidant, cytotoxic, and hypolipidemic). These chemical compounds are safe and there is no need for a withdrawal period when supplemented in the diet of chickens. The best result was obtained when date palm seed powder was supplemented at 400 g per kg diet compared to the other treatments. This level of supplementation poses no negative effect on the general performance and health status of broiler chickens. Further studies need to be

carried out to ascertain the effects of date palm seed powder on the histopathology of internal organs in chickens.

Declarations *Competing interests*

The author declared that there are no conflicts of interest.

Authors' contributions

Alagbe Olujimi John designed the experiment, carried out statistical analysis, and wrote the manuscript. The author read and approved the final version of the manuscript.

Authors' relationships and activities

All authors disclose any personal and financial relationships with other people or organizations.

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Availability of data and materials

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Ethical considerations

The author has reviewed all ethical problems, including plagiarism, consent to publish, data fabrication, and falsification.

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